

### Assessment of the risk of Hepatitis E virus occurrence in pork carcasses at slaughter in the UK.

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#### Abstract

Hepatitis E virus (HEV) is a RNA virus of the genus *Hepevirus*. HEV genotype 3 is zoonotic and pigs are the main reservoir. This genotype has been identified in the United Kingdom, in sporadic locally-acquired cases without recent history of foreign travel to endemic countries. The number of HEV-confirmed human cases in the UK has increased significantly, from 124 cases in 2003 to 661 in 2013. Non-travel cases now account for the majority (69%) of cases observed annually in the UK.

Our aim was to assess the risk of HEV occurrence in pork carcasses at slaughter in the UK. We performed a quantitative exposure assessment using Monte Carlo simulation to estimate the number of carcasses contaminated with HEV produced in a high throughput porcine abattoir during one year.

The input data used were: a) true prevalence in British pigs, b) probability of viral shedding in bile and faeces c) number of pig carcasses with bile and/or faecal contamination detected during post-mortem inspection. The following assumptions were made when data were unavailable: a) sensitivity and specificity of the RP-PCR diagnostic test; b) meat from viraemic pigs was considered HEV-positive; c) visual faecal and bile contamination only considered since microscopic contamination would not be detected through visual inspection.

We estimated through our model that 175,152 (2.4%) of carcasses produced in high throughput abattoirs would be infected with HEV in one year period. The number of viraemic pigs slaughtered at the abattoir was the largest driver of the uncertainty in carcass contamination. Variations in this parameter would change the output from 62,982 carcasses up to 306,320. Mitigation strategies at farm level should be explored, as it appears that control at this level would likely result in a higher reduction in HEV contamination in pork meat.

#### Introduction

Hepatitis E virus (HEV) is a RNA virus of the genus *Hepevirus*. In developed countries, HEV genotype 3 caused sporadic cases of Hepatitis E locally-acquired reported in individuals living in non-endemic areas, without history of recent travel. In the United Kingdom, the number of confirmed cases of HEV infection has increased significantly, from 124 cases in 2003 to 661 in 2013. Non-travel cases accounted for the 69% of HEV cases in 2013 (Public Health England, 2013).

The aim of the current study was to assess the presence of HEV in pork carcasses and identify potential sources for carcass contamination during slaughter. Sources of HEV assessed were viraemic pigs and faecal or bile contamination. In order to estimate the number of HEV-contaminated pig carcasses produced annually in a given UK abattoir, we performed a Quantitative Risk Assessment (QRA).

#### Materials and methods

A Quantitative Risk Assessment (QRA) following the Codex Alimentarius Commission requirements (Codex, 2009) was carried out to estimate how many pig carcasses produced, in a year, in a high throughput porcine

abattoir in the UK would be contaminated with Hepatitis E Virus using @Risk (ver. 6.3 Palisade Corporation) to develop a Monte Carlo simulation model. High throughput abattoirs were selected as these produce more than 100,000 heads per year, corresponding to 80% of the pork produced in the UK. This QRA is focused in intensive pig farming systems, as over 80% of the pork produced in UK is raised in this manner (BPEX and AHVLA).

### **Hazard identification**

Hepatitis E virus (HEV) is a positive sense, non-enveloped single-stranded RNA virus with a genome of 7.2 kb and can be grouped into at least four genotypes (Emerson S.U., 2003). At least two genotypes of swine HEV, genotypes 3 and 4, have been identified and characterized in pigs. Swine strains have often been related to human liver disease cases (Lu L, 2006). HEV investigations worldwide have been performed but there is still a lack of information on the infection dynamics in pig populations. It has been demonstrated that HEV, in most cases, causes only subclinical infections in pigs (Emerson SU, 2011). The recent study carried out by Public Health England (PHE) showed that 92.8% of the British finisher pigs, at slaughterhouse level, were HEV antibody-positive (Tedder, 2014).

### **Exposure assessment.**

A pathway was assessed to identify which steps of the slaughter process would need to be included in the model. The pathways considered were: a) Viraemic animals, b) Contamination from infected faeces, and c) Contamination from infected bile.

#### **3.2.1: Data source and parameters estimation:**

##### ***A) Probability of a finisher pig being viraemic ( $P_v$ ):***

PHE performed a survey on the 14 largest throughput abattoirs in the UK, which process 80% of pigs slaughtered in the UK. Six hundred twenty-nine pigs were tested for HEV using Real-Time PCR test (RT-PCR) and 36 pigs were found viraemic at slaughterhouse level. It was assumed that viraemic pigs would have HEV in their meat and blood, suggesting a potential role of blood contamination based on previous studies (Bouwknegt, 2009). An event tree was built to calculate the true prevalence considering the sensitivity ( $Se$ ) and specificity ( $Sp$ ) of RT-PCR test for RNA HEV.  $Se$  (98%) and  $Sp$  (96%) were assumed as data were not available. Bayesian Inference in a binomial process was used to estimate the prevalence of viraemic pigs ( $P_v$ ), with a uniform prior (1) and a probability mass function as a likelihood:

Then, posterior (prevalence) was calculated.

##### ***B) Probability of shedding HEV in faeces ( $P_{fj}$ ):***

For the purpose of our model, it was assumed that all viraemic pigs at slaughter shed the virus in the faeces (Bouwknegt, 2009), (Kanai, 2010). The estimation of the probability of any given finisher pig shedding virus in faeces was based in two published articles for the purpose of our QRA: 5 (11%) and 5 (12.5%) pigs were RNA positive in faeces out of 45 and 40 finisher pigs tested, respectively (McCreary, 2008) and (Berto, 2012). A Bayesian stochastic mathematical model was used to calculate the probability of a finisher pig is excreting the virus using McCreary's data as a prior and Berto's data as the likelihood.

## **C) Probability of having HEV in bile (Pbi):**

HEV multiplies in the liver and in consequence, presence of HEV in bile is very likely. Although in the UK, studies of HEV detection in bile have not been carried out, some data found in published articles in other countries (Italy and Spain) (Table 1).

Table 1. RT-PCR results for HEV in porcine bile.

Country	Number of Pigs	Number of pigs with RNA in bile	Author
Spain	26	20	N. de Deus et al. (2007)
Italy	137	41	F. Martelli et al. (2011)
Spain	80	5	M. Casas et al.(2011)
Italy	26	10	Di Bartolo et al.(2010)

A simplified conjugate prior was used, where  $\alpha$  and  $\beta$  are coming from the prior information and lead to another posterior beta distribution:

## **D & E) Probability of faecal contamination in carcasses (Pfc) & Probability of bile contamination in carcasses (Pbc):**

At the abattoirs, the evisceration point is the most critical control point for faecal and bile contamination in carcasses as this is very likely to occur through ruptured guts or the ruptured gall bladder during manual evisceration. Records of contamination at *post mortem* inspection (PMI) were used to calculate how many carcasses could have any faecal and bile contamination. It is assumed that if there is visual contamination there is also microscopic contamination by bacteria or viral particles. To calculate the probability of a carcass being either faecal or bile contaminated, a weighted beta distribution was used. The distribution was weighted by the number of animals killed per day. To avoid bias, the records were chosen from different abattoirs with high throughput (i.e., larger than 100,000 heads per year), five days a week, in particular the second week of every month, during first six months of the year.

## **F) Probability of any given carcass having any contamination of HEV**

The event trees were built to estimate:

Probability of any carcasses having the HEV in the meat. (A)=

Probability of any given carcass being contaminated with HEV from infected faecal contamination. (B)=

Probability of any given carcass being contaminated with HEV infected bile. (C) =

D. The probability addition rule was used to estimate the final probability where:

## **G) How many carcasses produced, in a high throughput abattoir from the UK, in a year period, will be contaminated with Hepatitis E Virus?**

The estimation of the number of carcasses produced that will have any HEV contamination of in a given year, in a high throughput abattoir from the UK was calculated using the formula:

Where  $\alpha$  was the number of pigs slaughtered in 2012 ( $n=7,209,342$ ), in high throughput abattoirs (87.7% of the annual porcine production in the UK) (AHDB/BPEX, 2012).  $P$  is the probability of any given carcass having any contamination of HEV.

## Results

The probability of a pig being viraemic ( $P_v$ ) followed a beta distribution with a mean of 2.02% (95% CI: 0.54% - 3.75%). The probability of shedding HEV in faeces ( $P_{fi}$ ), with a 95% confidence varied between 6.38% and 17.49% being the mean 11.53%. The probability of shedding HEV in bile ( $P_{bi}$ ) is 28.41% (23.98-33.75). Probability of faecal ( $P_{fc}$ ) and bile ( $P_{bc}$ ) contamination was 0.99% and 1.04% respectively. The probability of a pig shedding virus in faeces and having virus in bile is much higher than the probability of a carcass being viraemic. It was estimated through our model that, 26.39% (22.7-29.6) of non-viraemic HEV infected pigs would shed virus in bile and that 9.51% (5.4-15.2) of non-viraemic infected HEV pigs would excrete HEV in faeces. Therefore, taking into account the probability of faecal or bile contamination in carcasses, the overall probability of any given pig carcass having any contamination of HEV from viraemic animals, faecal and/or bile contamination is 2.4% (0.9%-4.3%). Most of the carcasses with HEV appeared to come from viraemic pigs; although the probability of having virus in faeces or in bile is much higher than the probability of being viraemic, the probability of faecal/ bile contamination is not as high and in consequence the probability of being viraemic contributed the most to the carcass contamination with HEV. The results show that in a year 175,152 (66,015-315,793) carcasses will have HEV out of the 7.2 million of carcasses produced.

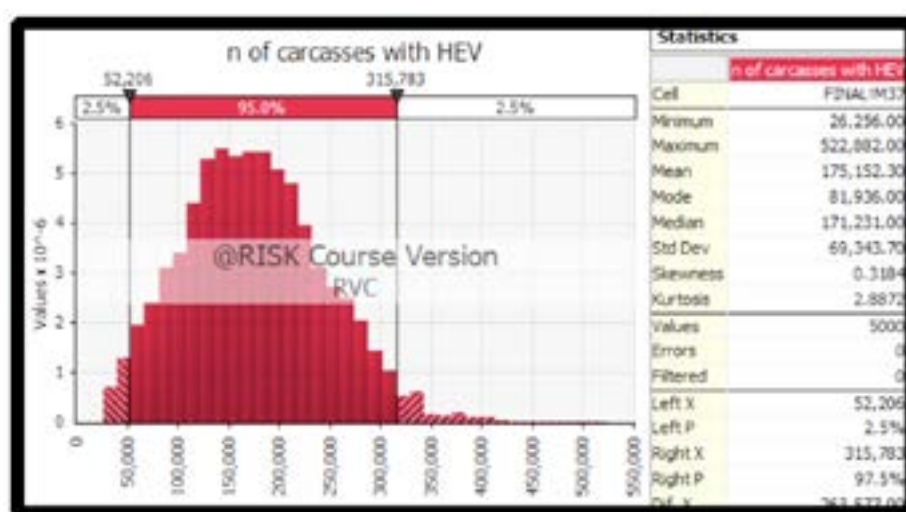


Figure 1. Final result: Number of carcasses with HEV

The Sensitivity Analysis showed that the number of viraemic pigs slaughtered at the abattoir was the largest driver of the uncertainty in carcass contamination. Variations in this parameter would change the output from 62,982 carcasses to 306,320.

## Discussion

Our results show that 175,152 pig carcasses will be infected with HEV, corresponding to 2.4% of the carcasses produced in high throughput pig abattoirs, in the UK, in a year period. The knowledge of the

number of carcasses that would cause illness in humans by a dose-response assessment would be desired. Our results showed that the major contribution for HEV-contaminated carcasses derived from viraemic pigs. Our model showed that non-viraemic pigs can have the virus in bile and faeces, thus HEV may also be in the meat of asymptomatic, infected animals. More studies seroprevalence studies virus in meat in viraemic and non viraemic pigs using serological and RT-RNA tests would be recommended to guide mitigation strategies at farm level. Other studies in weaner pig have shown that seropositive pigs can have detectable levels of HEV in liver, bile, faeces (Bouwknegt, 2009). The prevalence of seropositive pigs in the last study in the UK was 92.8% (Tedder, 2014), hence further studies are needed to determine how seropositive finisher pigs at the moment of slaughter behave shedding the virus. Berto *et al.* (2012) carried out a study of HEV in different points of the food chain and showed that six of 63 (9.5%) sausages were HEV positive (Berto, 2012). This corroborates that HEV can be present in the final product and pose a risk for the consumer; particularly, undercooked sausages could be one of the reasons of the recent significant increase of non-travel cases in the UK. This zoonosis usually only cause asymptomatic or mild symptoms in infected individuals, remaining the disease largely underreported. However, HEV can be fatal to immuno-depressed people and pregnant women.

Official certification of HEV free farm status could also be used to guarantee safe pork products. With certification of disease-free farms, a better knowledge of the farm prevalence would be gained in the UK. Furthermore, “all in all out” replacement procedures, the use of HEV-negative animals for breeding, cleansing and disinfection, good biosecurity practices of visitors, amongst other measures might reduce the prevalence of the virus in the farm. Sending animals for slaughtering after they are recovered from the viraemia might also decrease the number of contaminated carcasses. At food industry level, control of foodborne viruses is challenging. Cross-contamination could be avoided by preventing faecal and bile contamination avoiding rupture of guts or the gall bladder, cleaning and disinfection of surfaces and equipment during slaughter. The identification of HEV-positive batches before going to the abattoir and implementing measures to slaughter these the last in the day may also minimise the risk of cross-contamination. For consumers, public recommendation of good hygiene and cooking practices in the kitchen can also decrease the risk of HEV infection.

Lack of data was an issue and assumptions had to be made; for example Se and Sp of RT-PCR, although it was taken in consideration that the test had a very high sensitivity (Kanai, 2010). It was also assumed that viraemic pigs possess the virus in meat due to contamination from the blood (Bouwknegt, 2009). We considered only visual contamination; microscopic presence without visual contamination was not considered for the purpose of this study which could have led to underestimation of number of HEV-contaminated carcasses.

Taking into account the number of pigs produced in the UK in a high throughput abattoir, we concluded that 175,152 (66,015-315,793) pig carcasses produced, in a year, in a high throughput abattoir from the UK, will be HEV-positive with viraemic pigs being the main source of contamination. Further work is needed to assess and quantify the HEV contamination risk throughout the whole food chain.

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